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31	25	(biliverdin or biliverdine) adj reductase	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/10/27 17:15
37	0	((biliverdin or biliverdine) adj reductase) near5 actin	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/10/27 17:15
43	55080	cell near3 structure	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/10/27 17:16
49	1	((biliverdin or biliverdine) adj reductase) and (cell near3 structure)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/10/27 17:18
55	4969	cell near3 morphology	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/10/27 17:18
61	0	(cell near3 morphology) and ((biliverdin or biliverdine) adj reductase)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/10/27 17:18

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=> s l1 (3a) expression

23 FILES SEARCHED...

55 FILES SEARCHED...

L2 89 L1 (3A) EXPRESSION

=> s l2 and plant

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AN 2001:38818 AGRICOLA

DN IND22681159

TI Biliverdin reductase-induced phytochrome chromophore deficiency in transgenic tobacco.

AU Montgomery, B.L.; Franklin, K.A.; Terry, M.J.; Thomas, B.; Jackson, S.D.; Crepeau, M.W.; Lagarias, J.C.

AV DNAL (450 P692)

SO Plant physiology, Jan 2001. Vol. 125, No. 1. p. 266-277

Publisher: Rockville, MD : American Society of Plant Physiologists, 1926-CODEN: PLPHAY; ISSN: 0032-0889

NTE In the Special Issue: 75th Anniversary--Conceptual Breakthroughs in Biology.

Includes references

CY Maryland; United States

DT Article; Conference

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LA English

AB Targeted **expression** of mammalian **biliverdin IXalpha**

reductase (BVR), an enzyme that metabolically inactivates linear tetrapyrrole precursors of the phytochrome chromophore, was used to examine the physiological functions of phytochromes in the qualitative short-day tobacco (*Nicotiana tabacum* cv Maryland Mammoth) **plant**.

Comparative phenotypic and photobiological analyses of plastid- and cytosol-targeted BVR lines showed that multiple phytochrome-regulated processes, such as hypocotyl and internode elongation, anthocyanin synthesis, and photoperiodic regulation of flowering, were altered in all lines examined. The phytochrome-mediated processes of carotenoid and chlorophyll accumulation were strongly impaired in plastid-targeted lines, but were relatively unaffected in cytosol-targeted lines. Under certain growth conditions, plastid-targeted BVR expression was found to nearly abolish the qualitative inhibition of flowering by long-day photoperiods. The distinct phenotypes of the plastid-targeted BVR lines implicate a regulatory role for bilins in plastid development or, alternatively, reflect the consequence of altered tetrapyrrole metabolism in plastids due to bilin depletion.

L4 ANSWER 2 OF 5 AGRICOLA DUPLICATE 2
 AN 2000:40652 AGRICOLA
 DN IND22041274
 TI Modification of distinct aspects of photomorphogenesis via targeted **expression** of mammalian **biliverdin reductase** in transgenic *Arabidopsis* **plants**.
 AU Montgomery, B.L.; Yeh, K.C.; Crepeau, M.W.; Lagarias, J.C.
 CS University of California, Davis.
 AV DNAL (450 P692)
 SO Plant physiology, Oct 1999. Vol. 121, No. 2. p. 629-639
 Publisher: Rockville, MD : American Society of Plant Physiologists, 1926-
 CODEN: PLPHAY; ISSN: 0032-0889
 NTE Includes references
 CY Maryland; United States
 DT Article; Conference
 FS U.S. Imprints not USDA, Experiment or Extension
 LA English
 AB The phenotypic consequences of targeted **expression** of mammalian **biliverdin IXalpha reductase** (BVR), an enzyme that metabolically inactivates the linear tetrapyrrole precursors of the phytochrome chromophore, are addressed in this investigation. Through comparative phenotypic analyses of multiple plastid-targeted and cytosolic BVR transgenic *Arabidopsis* **plant** lines, we show that the sub-cellular localization of BVR affects distinct subsets of light-mediated and light-independent processes in **plant** growth and development. Regardless of its cellular localization, BVR suppresses the phytochrome-modulated responses of hypocotyl growth inhibition, sucrose-stimulated anthocyanin accumulation, and inhibition of floral initiation. By contrast, reduced protochlorophyll levels in dark-grown seedlings and fluence-rate-dependent reduction of chlorophyll occur only in transgenic **plants** in which BVR is targeted to plastids. Together with companion analyses of the phytochrome chromophore-deficient hyl mutant, our results suggest a regulatory role for linear tetrapyrroles within the plastid compartment distinct from their assembly with apophytocchromes in the cytosol.

L4 ANSWER 3 OF 5 AGRICOLA DUPLICATE 3
 AN 97:75514 AGRICOLA
 DN IND20598193
 TI Regulation of photomorphogenesis by **expression** of mammalian **biliverdin reductase** in transgenic *Arabidopsis* **plants**.
 AU Lagarias, D.M.; Crepeau, M.W.; Maines, M.D.; Lagarias, J.C.
 CS University of California-Davis, Davis, CA.
 SO The Plant cell, May 1997. Vol. 9, No. 5. p. 675-688
 Publisher: [Rockville, MD : American Society of Plant Physiologists, c1989-
 CODEN: PLCEEW; ISSN: 1040-4651
 NTE Includes references
 CY Maryland; United States

DT Article
FS U.S. Imprints not USDA, Experiment or Extension
LA English
AB The photoregulatory activity of the phytochrome photoreceptor requires the synthesis and covalent attachment of the linear tetrapyrrole prosthetic group phytochromobilin. Because the mammalian enzyme biliverdin IXalpha reductase (BVR) is able to functionally inactivate phytochromobilin in vitro, this investigation was undertaken to determine whether BVR expression in transgenic **plants** would prevent the synthesis of functionally active phytochrome in vivo. Here, we show that plastid-targeted, constitutive expression of BVR in Arabidopsis yields **plants** that display aberrant photomorphogenesis throughout their life cycle. Photobiological and biochemical analyses of three transgenic BVR lines exhibiting a 25-fold range of BVR expression established that the BVR-dependent phenotypes are light dependent, pleiotropic, and consonant with the loss of multiple phytochrome activities. Chlorophyll accumulation in BVR-expressing transgenic **plants** was particularly sensitive to increased light fluence rates, which is consistent with an important role for phytochrome in light tolerance. Under blue light, transgenic BVR **plants** displayed elongated hypocotyls but retained phototropic behavior and the ability to fully deetiolate. Directed BVR expression may prove to be useful for probing the cellular and developmental basis of phytochrome-mediated responses and for selective control of individual aspects of light-mediated **plant** growth and development.

L4 ANSWER 4 OF 5 AGRICOLA DUPLICATE 4
AN 94:34414 AGRICOLA
DN IND20389840
TI Inactivation of phytochrome- and phycobiliprotein-chromophore precursors by rat liver biliverdin reductase.
AU Terry, M.J.; Maines, M.D.; Lagarias, J.C.
AV DNAL (381 J824)
SO The Journal of biological chemistry, Dec 15, 1993. Vol. 268, No. 35. p. 26099-26106
Publisher: Baltimore, Md. : American Society for Biochemistry and Molecular Biology.
CODEN: JBCHA3; ISSN: 0021-9258
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AB The phytochrome chromophore precursor, 3E-phytochromobilin, and the phycobiliprotein chromophore precursors, 3E-phycocyanobilin and 3E-phycoerythrobilin, are enzymatically converted to novel rubinoid products by purified rat liver biliverdin reductase. Phytochromobilin and phycocyanobilin are particularly good substrates for biliverdin reductase with Km and Vmax values very similar to those of the natural substrate, biliverdin IX alpha. Phycoerythrobilin is the least preferred of the three bilin substrates. 1H NMR spectroscopy of phycocyanorubin, the product of phycocyanobilin catalysis by biliverdin reductase, and comparison of absorption spectra of all three rubinoid products reveal that the C10 methine bridge is selectively reduced by biliverdin reductase without altering the A-ring ethylidene substituent. In vitro phytochrome assembly experiments demonstrate that the phytorubin products do not form photoactive adducts with recombinant apophytochrome. These results suggest that ectopic **expression of biliverdin reductase in plants** will prevent assembly of the functional photoreceptor and thus will potentially alter light-mediated **plant** growth and development.

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 SUM 9503140. One part of this project addresses the biochemistry of key enzyme-mediated steps in the pathway of the phytochrome chromophore biosynthesis. A second part of this project initiates a novel experimental approach to alter **plant** photomorphogenesis by expression of a mammalian enzyme in transgenic **plants**. The long term goal of these investigations is to rationally alter the natural responses of **plants** to their light environment through modification of the synthesis and assembly of a functional phytochrome chromophore. Biochemical and molecular genetic approaches will be used to address the biochemistry of the two key enzymes of phytochrome chromophore biosynthesis, phytychromobilin synthase and the apaphytochrome molecule itself (i.e. phytychromobilin C-S lyase). Standard **plant** genetic transformation methodology will be used to construct transgenic **plants** expressing the chromophore-degrading enzyme biliverdin reductase in transgenic **plants**. PR assay for the penultimate enzyme of the biosynthetic pathway for the pigment moiety of the **plant** photoreceptor phytochrome that has enabled its biochemical characterization from both a **plant** and green algal source. The observation that native phytochrome from the green alga *Mesotaenium caldariorum* possesses a phycobilin prosthetic group has established a biochemical and evolutionary link between phytochrome and the light-harvesting photosynthetic antennae of red and blue-green algae. The ability to alter the natural responses of **plants** to their light environment via constitutive expression of the mammalian enzyme biliverdin reductase in transgenic *Arabidopsis* and *Nicotiana* species has been demonstrated. These studies strongly suggest that directed expression of BVR in **plants** will be useful for control of individual aspects of light-mediated **plant** growth and development. The development of improved yeast culture conditions for expression, assembly and purification of recombinant phytochromes has also enabled us to prepare and characterize the unnatural, yellow-orange fluorescent bilin adducts of oat, green algal and cyanobacterial apophytochromes. We have shown that these adducts, that we have named phytofluors, are intensely fluorescent, photostable and chemically stable proteins that can be reconstituted in living cells. PB natural chromophore precursor of phytochrome in the green alga *Mesotaenium caldariorum*. J. Biol. Chem. 272, 25700-25705. PB Regulation of photomorphogenesis by **expression** of mammalian **biliverdin reductase** in transgenic *arabidopsis* **plants**. **Plant** Cell 9, PB of recombinant affinity peptide-tagged oat phytochrome A. Photochem. Photobiol. 65, 750-758. PB fluorescent protein probes. Current Biology, 7: 870-876. PB green alga *Mesotaenium caldariorum*. Implication for a conserved mechanism of phytochrome action. **Plant** Cell Environ. 20, 691-699.

=>

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L1 1366 (BILIVERDIN OR BILIBERDINE) (W) REDUCTASE

=> s cell (3a) (structure or morphology)

11 FILES SEARCHED...

13 FILES SEARCHED...

23 FILES SEARCHED...

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L2 340021 CELL (3A) (STRUCTURE OR MORPHOLOGY)

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68 FILES SEARCHED...

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L3 57974 (CELL'S OR CELLS' OR CELLULAR) (3A) (STRUCTURE OR MORPHOLOGY)

=> s l2 or l3

51 FILES SEARCHED...

L4 387123 L2 OR L3

=> s (biliverdin or biliverdine) (w) reductase

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L7 ANSWER 1 OF 2 USPATFULL

AN 2002:171871 USPATFULL

TI Identification of drugs and drug targets by detection of the stress response

IN Davis, Ronald W., Palo Alto, CA, UNITED STATES

Giaever, Guri N., Palo Alto, CA, UNITED STATES

PI US 2002090620 A1 20020711

AI US 2001-898745 A1 20010703 (9)

PRAI US 2000-218288P 20000714 (60)

DT Utility

FS APPLICATION

LREP Carol L. Francis, Bozicevic, Field and Francis LLP, Suite 200, 200 Middlefield Road, Menlo Park, CA, 94025

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 1 Drawing Page(s)

LN.CNT 1530

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention features methods of high throughput screening of candidate drug agents and rapid identification of drug targets by examining induction of the stress response in a host cell, e.g., the stress response in wildtype host cells and/or in host cells that differ in target gene product dosage (e.g., host cells that have two copies of a drug target gene product-encoding sequence relative to one copy). In general, induction of the stress response in wildtype host cells indicates that a candidate agent has activity of the drug. Induction of a relatively lower or undetectable stress response in a host cell comprising an alteration in gene product dosage indicates that the host cell is drug-sensitive and is altered in a gene product that plays a role in resistance to the drug.

L7 ANSWER 2 OF 2 USPATFULL

AN 2002:55155 USPATFULL

TI Human single nucleotide polymorphisms

IN Cargill, Michele, Gaithersburg, MD, UNITED STATES

Ireland, James S., Gaithersburg, MD, UNITED STATES

Lander, Eric S., Cambridge, MA, UNITED STATES

PA Whitehead Institute for Biomedical Research, Cambridge, MA, UNITED STATES (U.S. corporation)

PI US 2002032319 A1 20020314

AI US 2001-801274 A1 20010307 (9)

PRAI US 2000-187510P 20000307 (60)

US 2000-206129P 20000522 (60)

DT Utility

FS APPLICATION
LREP HAMILTON BROOK SMITH AND REYNOLDS, P.C., TWO MILITIA DR, LEXINGTON, MA,
02421-4799
CLMN Number of Claims: 12
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 8981

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides nucleic acid segments of the human genome, particularly nucleic acid segments from genes including polymorphic sites. Allele-specific primers and probes hybridizing to regions flanking or containing these sites are also provided. The nucleic acids, primers and probes are used in applications such as phenotype correlations, forensics, paternity testing, medicine and genetic analysis.

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=> s 15 (3A) transfection

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=> s 15 (3A) expression

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L11 70 L5 (3A) EXPRESSION

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Mitsumasa; Shioiri, Hiroki; Sato, Ko-ichi; Kitamuro, Tomomi; Shirato,
Kunio; Yamaguchi, Tokio; Suematsu, Makoto; Shibahara, Shigeki (1)
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SO Arteriosclerosis Thrombosis and Vascular Biology, (August, 2001) Vol. 21,
No. 8, pp. 1373-1377. print.
ISSN: 1079-5642.
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LA English
SL English
AB Heme oxygenase-1 (HO-1) catalyzes the regiospecific oxidative degradation
of heme to biliverdin IXalpha, iron, and carbon monoxide. Biliverdin
IXalpha is subsequently reduced to bilirubin IXalpha by **biliverdin
reductase**. HO-1 **expression** is induced under various
disease conditions, including atherosclerosis, but it is unknown whether
HO-1 catalyzes heme breakdown in the regions at risk. Using
hypercholesterolemic rabbits fed a cholesterol-enriched diet, we attempted
to demonstrate the involvement of HO-1 induction and bilirubin IXalpha
production in atherosclerotic regions. Expression levels of HO-1 mRNA were
elevated in the aortas of hypercholesterolemic rabbits. In situ
hybridization and immunohistochemistry revealed that mRNA and protein of
HO-1 are induced in endothelial cells and foam cells (lipid-filled
macrophages) in atherosclerotic lesions. Furthermore, immunohistochemistry
with the use of an anti-bilirubin-IXalpha monoclonal antibody, 24G7,
demonstrated accumulation of bilirubin IXalpha in foam cells, indicating
that heme is actually degraded in atherosclerotic lesions. Remarkably,
bilirubin IXalpha, like HO-1 protein, is predominantly accumulated in the
perinuclear regions of foam cells. These results provide the first in vivo
evidence of the colocalization of HO-1 and bilirubin IXalpha in foam
cells, suggesting a role of HO-1 induction in the modulation of macrophage
activation in atherosclerosis.
- L12 ANSWER 2 OF 13 CAPLUS COPYRIGHT 2002 ACS
AN 2001:378600 CAPLUS
DN 135:134739
TI Biliverdin reductase-induced phytochrome chromophore deficiency in
transgenic tobacco
AU Montgomery, Beronda L.; Franklin, Keara A.; Terry, Matthew J.; Thomas,
Brian; Jackson, Stephen D.; Crepeau, Marc W.; Lagarias, J. Clark
CS Section of Molecular and Cellular Biology, University of California,
Davis, CA, 95616, USA
SO Plant Physiology (2001), 125(1), 266-277
CODEN: PLPHAY; ISSN: 0032-0889
PB American Society of Plant Physiologists
DT Journal
LA English
AB Targeted expression of mammalian biliverdin IX.alpha. reductase (BVR), an
enzyme that metabolically inactivates linear tetrapyrrole precursors of
the phytochrome chromophore, was used to examine the physiol. functions of
phytochromes in the qual. short-day tobacco (Nicotiana tabacum cv Maryland
Mammoth) plant. Comparative phenotypic and photobiol. analyses of

plastid- and cytosol-targeted BVR lines showed that multiple phytochrome-regulated processes, such as hypocotyl and internode elongation, anthocyanin synthesis, and photoperiodic regulation of flowering, were altered in all lines examd. The phytochrome-mediated processes of carotenoid and chlorophyll accumulation were strongly impaired in plastid-targeted lines, but were relatively unaffected in cytosol-targeted lines. Under certain growth conditions, plastid-targeted BVR expression was found to nearly abolish the qual. inhibition of flowering by long-day photoperiods. The distinct phenotypes of the plastid-targeted BVR lines implicate a regulatory role for bilins in plastid development or, alternatively, reflect the consequence of altered tetrapyrrole metab. in plastids due to bilin depletion.

RE.CNT 47 THERE ARE 47 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 3 OF 13 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
2
AN 1999:481091 BIOSIS
DN PREV199900481091
TI The oxidoreductase, biliverdin reductase, is induced in human renal carcinoma - pH and cofactor-specific increase in activity.
AU Maines, Mahin D. (1); Mayer, Robert D.; Erturk, Erdal; Huang, Tian J.; Disantagnese, Anthony
CS (1) Department of Biochemistry/Biophysics, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, NY, 14642 USA
SO Journal of Urology, (Oct., 1999) Vol. 162, No. 4, pp. 1467-1472. ISSN: 0022-5347.
DT Article
LA English
SL English
AB Purpose: Biliverdin reductase is an oxidoreductase unique among all enzymes characterized to date in having dual pH/dual cofactor requirement - NADH and NADPH at 6.7 and 8.7, respectively. The protein shows extensive microheterogeneity that is caused by post-translational modification. The reductase converts the heme degradation product, biliverdin, to bilirubin. Bilirubin has been shown to inhibit responses of human lymphocytes, including phytohemagglutinin-induced proliferation, interleukin-2 production, and antibody dependent and independent cell mediated cytotoxicity. In addition to acting as an antioxidant, it inhibits protein phosphorylation and activity of enzymes such as protein kinase C and NADPH oxidase. This research was to evaluate whether renal cell carcinoma differs from normal tissue in regard to the expression and activity of the reductase. Materials and Methods: kidney tissue with or without visible renal carcinoma and normal kidney tissue from a brain dead patient were frozen at -80C shortly after removal. Ten mum. tissue sections were used for immunostaining of biliverdin reductase, pooled isolated tumors and surrounding tissue that did not contain visible tumor were used for Northern blot analysis of mRNA and Western blot analysis of protein. Enzyme activity was also measured in these preparations at pH 6.7 with NADH, and at pH 8.7 with NADPH. Ten additional formalin fixed specimens of renal cell carcinoma were also used for immunostaining. Results: There was a striking increase in the reductase protein levels, as visualized by immunostaining in tumor tissue cells. The increase was also evident by Western blotting, and involved in increased transcription of biliverdin reductase as suggested by Northern blot analysis. The protein would also be detected in the infiltrating monocytes, macrophages, T cells and neutrophils as well as in circulating lymphocytes. The enzyme activity was nearly doubled in the tumor tissue, but selectively with NADH as the cofactor. Conclusion: Increases in **biliverdin reductase expression** and activity only with NADH is found in renal cell carcinoma. The net effects of this change are uncertain at present but several pathways, which could be affected by the reductase, may alter

local physiology. Biliverdin reductase as a zinc metalloprotein may directly interact with other regulatory proteins, generation of increased bilirubin may alter immune function and increased enzyme activity may deplete NADH with contrasting consequence of blocking free radical formation and depleting cellular ATP. To the benefit of the host, the latter could culminate in tumor cell death.

L12 ANSWER 4 OF 13 CAPLUS COPYRIGHT 2002 ACS

AN 1999:793142 CAPLUS

DN 132:135239

TI Heme oxygenase-1, heme oxygenase-2 and biliverdin reductase in peripheral ganglia from rat, expression and plasticity

AU Magnusson, S.; Ekstrom, J.; Elmer, E.; Kanje, M.; Ny, L.; Alm, P.

CS Department of Animal Physiology, Lund University, Lund, Swed.

SO Neuroscience (Oxford) (1999), Volume Date 2000, 95(3), 821-829

CODEN: NRSCDN; ISSN: 0306-4522

PB Elsevier Science Ltd.

DT Journal

LA English

AB The expression of inducible and constitutive heme oxygenase isoforms (HO-1 and HO-2, resp.) and biliverdin reductase (BVR) was studied in normal and cultured peripheral ganglia from adult rats, using immunocytochem. and in-situ hybridization. Dramatic changes were induced by 1-2 days' culturing of dorsal root ganglia, nodose ganglia, otic ganglia, sphenopalatine ganglia, and superior cervical ganglia. An up-regulation of HO-1 was found in satellite cells of the cultured nodose ganglia, dorsal root ganglia, sphenopalatine ganglia, and otic ganglia, whereas only a few satellite cells in the superior cervical ganglia responded with an increase in HO-1 immunoreactivity. In the superior cervical ganglia, HO-1 also appeared in a subpopulation of macrophages. During culturing, expression of HO-1 immunoreactivity also increased in axons and in nerve cell bodies. In-situ hybridization corroborated the immunocytochem. findings, revealing a strong up-regulation of HO-1 mRNA in satellite cells, and less pronounced up-regulation in nerve cell bodies. HO-2 immunoreactivity was found in most neurons in all of the ganglia studied. No significant changes in HO-2 immunoreactivity could be obsd. in cultured ganglia. BVR immunoreactivity was barely detectable in any of the normal ganglia; however, after culturing it appeared in axons, single nerve cell bodies, and nerve cell nuclei. The results showed that HO-1 is up-regulated in peripheral ganglia after axonal injury, and suggest a role for CO in cellular signaling and a requirement for the antioxidant, bilirubin, during the regeneration process.

RE.CNT 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 5 OF 13 AGRICOLA

DUPLICATE 3

AN 2000:40652 AGRICOLA

DN IND22041274

TI Modification of distinct aspects of photomorphogenesis via targeted **expression** of mammalian **biliverdin reductase** in transgenic Arabidopsis plants.

AU Montgomery, B.L.; Yeh, K.C.; Crepeau, M.W.; Lagarias, J.C.

CS University of California, Davis.

AV DNAL (450 P692)

SO Plant physiology, Oct 1999. Vol. 121, No. 2. p. 629-639

Publisher: Rockville, MD : American Society of Plant Physiologists, 1926-

CODEN: PLPHAY; ISSN: 0032-0889

NTE Includes references

CY Maryland; United States

DT Article; Conference

FS U.S. Imprints not USDA, Experiment or Extension

LA English

AB The phenotypic consequences of targeted expression of mammalian biliverdin IX α reductase (BVR), an enzyme that metabolically inactivates the linear tetrapyrrole precursors of the phytochrome chromophore, are addressed in this investigation. Through comparative phenotypic analyses of multiple plastid-targeted and cytosolic BVR transgenic Arabidopsis plant lines, we show that the sub-cellular localization of BVR affects distinct subsets of light-mediated and light-independent processes in plant growth and development. Regardless of its cellular localization, BVR suppresses the phytochrome-modulated responses of hypocotyl growth inhibition, sucrose-stimulated anthocyanin accumulation, and inhibition of floral initiation. By contrast, reduced protochlorophyll levels in dark-grown seedlings and fluence-rate-dependent reduction of chlorophyll occur only in transgenic plants in which BVR is targeted to plastids. Together with companion analyses of the phytochrome chromophore-deficient hyl mutant, our results suggest a regulatory role for linear tetrapyrroles within the plastid compartment distinct from their assembly with apophytocchromes in the cytosol.

L12 ANSWER 6 OF 13 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
4

AN 2000:56492 BIOSIS

DN PREV200000056492

TI Enhanced neuronal **expression** of the oxidoreductase -
biliverdin reductase - after permanent focal cerebral
ischemia.

AU Panahian, Nariman; Huang, Tianjun; Maines, Mahin D. (1)

CS (1) Departments of Biochemistry and Biophysics, University of Rochester
School of Medicine, 601 Elmwood Ave., Rochester, NY USA

SO Brain Research, (Dec. 11, 1999) Vol. 850, No. 1-2, pp. 1-13.
ISSN: 0006-8993.

DT Article

LA English

SL English

AB This is the first report on increased neuronal levels of biliverdin reductase (BVR) in response to ischemic brain injury. BVR is an oxidoreductase, and is unique among all enzymes characterized to date in having dual pH/dual cofactor requirements - NADH and NADPH at 6.7 and 8.7, respectively. BVR catalyses the final step in the heme metabolic pathway and reduces the heme degradation product, biliverdin, to bilirubin. Bilirubin can be both a neurotoxicant and an antioxidant depending on its ratio to protein and concentration. Bilirubin also has immunomodulatory activity. Other biologically active heme degradation products are iron and CO. This study assessed time-dependent changes in the level of BVR, following permanent middle cerebral artery occlusion (MCAo). It also examined correlation of the change in BVR expression with display of indices of ischemic tissue injury. Under halothane anesthesia and normothermic conditions, 72 DNX inbred mice were subjected to MCAo. A time-dependent enlargement of an ischemic lesion over the course of 24 h was observed and measured 55 \pm 5 mm³ at 6 h, 63 \pm 6.7 mm³ at 12 h, and 73 \pm 5 mm³ at 24 h. Six hours after MCAo, increased immunoreactivity for BVR was noted in neurons in the peri-ischemic areas, intraischemic cortical layers 3 and 5, as well as in neurons in regions distant from the borders of vascular distribution of the MCA, such as those in substantia nigra, in the Purkinje layer of the cerebellum and in the central nucleus of inferior colliculus. Twenty-four hours after MCAo, immunoreactivity for BVR remained increased in the peri-ischemia areas. At all time points staining for BVR was decreased in the ischemic core. At the 24 h time point there was an increase in Fe staining in the perimeter of the lesion and an increase in Schiff's staining for lipid peroxidation at the rim of the lesion. In situ hybridization analysis demonstrated a time dependent increase in BVR mRNA labeling in neurons of the peri-ischemic area. In the ischemic hemisphere, when compared with the contralateral hemisphere,

neither measurable decreases in BVR mRNA or total protein levels nor a decrease in NADH-dependent BVR activity at pH 6.7 were observed. As judged by Northern and Western blots and activity analysis, despite the apparent loss of BVR from the ischemic core, and its increase in the peri-ischemic region, when compared with the contralateral hemisphere, the overall capacity of the ischemic hemisphere to catalyze the reduction of biliverdin was unchanged throughout the experiment. Should, in the case of ischemia, the conditions favor the antioxidant activity of bilirubin, then we suggest that increase in BVR expression in ischemic penumbra may present a cellular defense mechanism against free radical-mediated neuronal damage. Furthermore, we interpret the apparent tightly regulated expression of BVR in the ischemic hemisphere as an important factor in protection against bilirubin neurotoxicity. Data suggest that pharmacological modulation of BVR expression is a possible new direction for protecting neurons against ischemic injury and oxidative stress.

L12 ANSWER 7 OF 13 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
5

AN 1998:270178 BIOSIS

DN PREV199800270178

TI A chromatographic assay for heme oxygenase activity in cultured human cells: Application to artificial heme oxygenase overexpression.

AU Ryter, Stefan; Kvam, Egil; Richman, Larry; Hartmann, Francoise; Tyrrell, Rex M. (1)

CS (1) Univ. Bath, Sch. Pharmacy and Pharmacol., Bath BA2 7AY UK

SO Free Radical Biology & Medicine, (April, 1998) Vol. 24, No. 6, pp. 959-971.

ISSN: 0891-5849.

DT Article

LA English

AB Heme oxygenase (HO) activity oxidizes heme, releasing carbon monoxide; heme iron; and biliverdin, which is converted to bilirubin by

biliverdin reductase. Inducible HO-I expression

is a marker of oxidative stress in mammalian cells, while noninducible HO-II contributes to basal HO activity. HO-I and HO-II activities are implicated in cellular antioxidant defense mechanisms. We describe a microassay for HO activity in cultured human cells, using high-performance liquid chromatography of biliverdin and bilirubin. The assay is sufficiently sensitive to quantify basal and inducible HO activity in various human cell types. We have established human cell lines overexpressing heme oxygenase-II activity in microsomes using a metallothionein promoter-regulated expression system. Stable transformants treated with ZnCl₂ express up to ninefold induction of HO activity. We have constructed human cell lines overexpressing HO-II protein and activity (5-15-fold) in the absence of tetracycline, using the HtTA-1 cell line transfected with tetracycline-regulated expression vectors (Gossen et al., Proc. Natl. Acad. Sci. USA 89, 1992). Functional HO-II overexpressing clones will be useful in investigating anti- or pro-oxidant effects of HO activity during cellular oxidative stress.

L12 ANSWER 8 OF 13 CAPLUS COPYRIGHT 2002 ACS

AN 1997:351213 CAPLUS

DN 127:30735

TI Characterization of cyanobacterial biliverdin reductase. Conversion of biliverdin to bilirubin is important for normal phycobiliprotein biosynthesis

AU Schluchter, Wendy M.; Glazer, Alexander N.

CS Department Molecular Cell Biology, University California, Berkeley, CA, 94720-3206, USA

SO Journal of Biological Chemistry (1997), 272(21), 13562-13569

CODEN: JBCHA3; ISSN: 0021-9258

PB American Society for Biochemistry and Molecular Biology

DT Journal
LA English
AB The *Synechocystis* sp. PCC 6803 gene (bvdR) encoding biliverdin reductase (I) was amplified by the polymerase chain reaction, cloned, and overexpressed in *Escherichia coli* as the native form and as a 6-histidine-tagged N-terminal fusion. The latter form of I was purified by affinity chromatog. and shown to have the appropriate mol. wt. by electrospray mass spectrometry. Both forms of I reduced biliverdin IX.alpha. using NADPH or NADH, with NADPH as the preferred reductant. His-tagged I had a K_m for biliverdin of 1.3 μ M. The pH optimum for the NADPH-dependent activity was 5.8, whereas that for rat I is at pH 8.7. Absorbance spectra and HPLC retention times of the reaction product reaction matched those of authentic bilirubin, the product of the redn. of biliverdin by the mammalian enzymes. These results provide the 1st evidence for the formation of bilirubin in bacteria. Fully segregated *Synechocystis* sp. PCC 6803 bvdR interposon mutants produced .apprx.85% of the normal amt. of phycobilisome cores contg. allophycocyanin and other phycocyanobilin-bearing core polypeptides, but no detectable phycocyanin. Thus, surprisingly, the blockage of the conversion of biliverdin to bilirubin interferes with normal phycobiliprotein biosynthesis in cyanobacteria. Possible interpretations of this finding are presented.

L12 ANSWER 9 OF 13 AGRICOLA DUPLICATE 6

AN 97:75514 AGRICOLA

DN IND20598193

TI Regulation of photomorphogenesis by **expression** of mammalian **biliverdin reductase** in transgenic *Arabidopsis* plants.

AU Lagarias, D.M.; Crepeau, M.W.; Maines, M.D.; Lagarias, J.C.

CS University of California-Davis, Davis, CA.

SO The Plant cell, May 1997. Vol. 9, No. 5. p. 675-688

Publisher: [Rockville, MD : American Society of Plant Physiologists, c1989-

CODEN: PLCEEW; ISSN: 1040-4651

NTE Includes references

CY Maryland; United States

DT Article

FS U.S. Imprints not USDA, Experiment or Extension

LA English

AB The photoregulatory activity of the phytochrome photoreceptor requires the synthesis and covalent attachment of the linear tetrapyrrole prosthetic group phytychromobilin. Because the mammalian enzyme biliverdin IXalpha reductase (BVR) is able to functionally inactivate phytychromobilin in vitro, this investigation was undertaken to determine whether BVR expression in transgenic plants would prevent the synthesis of functionally active phytyochrome in vivo. Here, we show that plastid-targeted, constitutive expression of BVR in *Arabidopsis* yields plants that display aberrant photomorphogenesis throughout their life cycle. Photobiological and biochemical analyses of three transgenic BVR lines exhibiting a 25-fold range of BVR expression established that the BVR-dependent phenotypes are light dependent, pleiotropic, and consonant with the loss of multiple phytyochrome activities. Chlorophyll accumulation in BVR-expressing transgenic plants was particularly sensitive to increased light fluence rates, which is consistent with an important role for phytyochrome in light tolerance. Under blue light, transgenic BVR plants displayed elongated hypocotyls but retained phototropic behavior and the ability to fully deetiolate. Directed BVR expression may prove to be useful for probing the cellular and developmental basis of phytyochrome-mediated responses and for selective control of individual aspects of light-mediated plant growth and development.

L12 ANSWER 10 OF 13 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE

AN 1995:519560 BIOSIS
 DN PREV199598533860
 TI **Expression of rat biliverdin reductase as a glutathione-S-transferase fusion protein.**
 AU Ennis, Orla M.; Mantle, Tim
 CS Dep. Biochem., Trinity College, Dublin 2 Ireland
 SO Biochemical Society Transactions, (1995) Vol. 23, No. 3, pp. 443S.
 Meeting Info.: 654th Meeting of the Biochemical Society Leicester, England, UK April 4-7, 1995
 ISSN: 0300-5127.
 DT Conference
 LA English

L12 ANSWER 11 OF 13 AGRICOLA DUPLICATE 8
 AN 94:34414 AGRICOLA
 DN IND20389840
 TI Inactivation of phytochrome- and phycobiliprotein-chromophore precursors by rat liver biliverdin reductase.
 AU Terry, M.J.; Maines, M.D.; Lagarias, J.C.
 AV DNAL (381 J824)
 SO The Journal of biological chemistry, Dec 15, 1993. Vol. 268, No. 35. p. 26099-26106
 Publisher: Baltimore, Md. : American Society for Biochemistry and Molecular Biology.
 CODEN: JBCHA3; ISSN: 0021-9258
 NTE Includes references
 CY Maryland; United States
 DT Article
 FS U.S. Imprints not USDA, Experiment or Extension
 LA English
 AB The phytochrome chromophore precursor, 3E-phytochromobilin, and the phycobiliprotein chromophore precursors, 3E-phycoerythrobilin and 3E-phycoerythrobilin, are enzymatically converted to novel rubinoid products by purified rat liver biliverdin reductase. Phytochromobilin and phycocyanobilin are particularly good substrates for biliverdin reductase with Km and Vmax values very similar to those of the natural substrate, biliverdin IX alpha. Phycoerythrobilin is the least preferred of the three bilin substrates. 1H NMR spectroscopy of phycocyanorubin, the product of phycocyanobilin catalysis by biliverdin reductase, and comparison of absorption spectra of all three rubinoid products reveal that the C10 methine bridge is selectively reduced by biliverdin reductase without altering the A-ring ethylidene substituent. In vitro phytochrome assembly experiments demonstrate that the phytorubin products do not form photoactive adducts with recombinant apophytochrome. These results suggest that ectopic **expression of biliverdin reductase** in plants will prevent assembly of the functional photoreceptor and thus will potentially alter light-mediated plant growth and development.

L12 ANSWER 12 OF 13 CAPLUS COPYRIGHT 2002 ACS
 AN 1990:628435 CAPLUS
 DN 113:228435
 TI Multiple forms of biliverdin reductase: age-related change in pattern of expression in rat liver and brain
 AU Maines, Mahin D.
 CS Sch. Med., Univ. Rochester, Rochester, NY, 14642, USA
 SO Molecular Pharmacology (1990), 38(4), 481-5
 CODEN: MOPMA3; ISSN: 0026-895X
 DT Journal
 LA English
 AB The present study reports on the tissue-dependent pattern of developmental **expression of the biliverdin reductase** in rat

liver and brain. When analyzed by Western immunoblotting, 2 closely migrating immunoreactive proteins were detected in the liver cytosol during the 1st 2-3 wk after birth; the protein with greater mobility was not detected in the liver of adult aged animals (6 mo old) and was present at low levels in rats during the 1st week of life. The faster migrating protein was not detected in the brain cytosol at any stage of development. Furthermore, in the brain the total amt. of enzyme protein increased as the animal matured, whereas in the liver the enzyme protein level decreased with age. When the purified enzyme was analyzed, age-related changes in the variant compn. of the enzyme in the liver were noted. Although in both adult and newborn animals (14 days old) the purified enzyme, when subjected to isoelec. focusing, separates into 5 net charge forms (pI 6.23, 5.91, 5.76, 5.61, and 5.48), the relative abundance of the variants was notably different in the 2 preps. In addn., when the purified preps. were subjected to 2-dimensional electrophoresis, although both purified preps. sep. into 3 mol. wt. forms (Mr 30,400, 30,700, and 31,400), 1 species (Mr 31,400, pI = 5.77), which was very prominently expressed in the newborn, was essentially absent in the adult. Biliverdin reductase activity of the liver cytosol with both NADPH (pH 8.7) and NADH (pH 6.7) exhibited developmental changes, with the activity increasing after birth, reaching a peak on day 14, and decreasing to low levels in the adult. The existence of a close correlation between development of biliverdin reductase activity in the brain and liver and that of heme oxygenase in these organs is noted. The suggestion is made that the reductase is not a passive component of the heme degrdn. pathway; rather, its activity could become limiting in the elimination of heme degrdn. products.

L12 ANSWER 13 OF 13 FEDRIP COPYRIGHT 2002 NTIS
 AN 2002:105149 FEDRIP
 NR AGRIC 0169655
 TI PHYTOCHROME CHROMOPHORE BIOSYNTHESIS & ITS REGULATION IN TRANSGENIC PLANTS
 SF LaGarias, J. C.
 CSP UNIV OF CALIFORNIA, MOLECULAR & CELLULAR BIOLOGY, DAVIS, CALIFORNIA, 95616
 FU NRI COMPETITIVE GRANT |c C
 FS Department of Agriculture
 SUM 9503140. One part of this project addresses the biochemistry of key enzyme-mediated steps in the pathway of the phytochrome chromophore biosynthesis. A second part of this project initiates a novel experimental approach to alter plant photomorphogenesis by expression of a mammalian enzyme in transgenic plants. The long term goal of these investigations is to rationally alter the natural responses of plants to their light environment through modification of the synthesis and assembly of a functional phytochrome chromophore. Biochemical and molecular genetic approaches will be used to address the biochemistry of the two key enzymes of phytochrome biosynthesis, phytychromobilin synthase and the apaphytochrome molecule itself (i.e. phytychromobilin C-S lyase). Standard plant genetic transformation methodology will be used to construct transgenic plants expressing the chromophore-degrading enzyme biliverdin reductase in transgenic plants. PR assay for the penultimate enzyme of the biosynthetic pathway for the pigment moiety of the plant photoreceptor phytochrome that has enabled its biochemical characterization from both a plant and green algal source. The observation that native phytochrome from the green alga *Mesotaenium caldariorum* possesses a phycobilin prosthetic group has established a biochemical and evolutionary link between phytochrome and the light-harvesting photosynthetic antennae of red and blue-green algae. The ability to alter the natural responses of plants to their light environment via constitutive expression of the mammalian enzyme biliverdin reductase in transgenic *Arabidopsis* and *Nicotiana* species has been demonstrated. These studies strongly suggest that directed expression of BVR in plants will be useful for control of individual aspects of light-mediated plant growth and development. The

development of improved yeast culture conditions for expression, assembly and purification of recombinant phytochromes has also enabled us to prepare and characterize the unnatural, yellow-orange fluorescent bilin adducts of oat, green algal and cyanobacterial apophytochromes. We have shown that these adducts, that we have named phytofluors, are intensely fluorescent, photostable and chemically stable proteins that can be reconstituted in living cells. PB natural chromophore precursor of phytochrome in the green alga *Mesotaenium caldariorum*. J. Biol. Chem. 272, 25700-25705. PB Regulation of photomorphogenesis by **expression** of mammalian **biliverdin reductase** in transgenic arabidopsis plants. Plant Cell 9, PB of recombinant affinity peptide-tagged oat phytochrome A. Photochem. Photobiol. 65, 750-758. PB fluorescent protein probes. Current Biology, 7: 870-876. PB green alga *Mesotaenium caldariorum*. Implication for a conserved mechanism of phytochrome action. Plant Cell Environ. 20, 691-699.

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